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Changes in Biochemical Composition of the Cell Wall of the Cotton Fiber During Development[1]

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ABSTRACT

The composition of the cell wall of the cotton fiber (*Gossypium hirsutum* L. Acala SJ-1) has been studied from the early stages of elongation (5 days postanthesis) through the period of secondary wall formation, using cell walls derived both from fibers developing on the plant and from fibers obtained from excised, cultured ovules. The cell wall of the elongating cotton fiber was shown to be a dynamic structure. Expressed as a weight per cent of the total cell wall, cellulose, neutral sugars (rhamnose, fucose, arabinose, mannose, galactose, and noncellulosic glucose), uronic acids, and total protein undergo marked changes in content during the elongation period. As a way of analyzing absolute changes in the walls with time, data have also been expressed as grams component per millimeter of fiber length. Expressed in this way for plant-grown fibers, the data show that the thickness of the cell wall is relatively constant until about 12 days postanthesis; after this time it markedly increases until secondary wall cellulose deposition is completed. Between 12 and 16 days postanthesis increases in all components contribute to total wall increase per millimeter fiber length. The deposition of secondary wall cellulose begins at about 16 days postanthesis (at least 5 days prior to the cessation of elongation) and continues until about 32 days postanthesis. At the time of the onset of secondary wall cellulose deposition, a sharp decline in protein and uronic acid content occurs. The content of some of the individual neutral sugars changes during development, the most prominent change being a large increase in noncellulosic glucose which occurs just prior to the onset of secondary wall cellulose deposition. Methylation analyses indicate that this glucose, at least in part, is 3-linked. In contrast to the neutral sugars, no significant changes in cell wall amino acid composition are observed during fiber development.

Compositional analyses of cell walls derived from culture-grown fibers indicate that these walls are remarkably similar to those derived from fibers grown on the plant, both in terms of composition and in terms of relative changes in composition during development.

A comparison of our results on total cell wall composition and linkages of sugars as determined by a preliminary methylation analysis of unfractionated fiber walls indicates that the primary cell wall of cotton fibers is similar to that of primary cell walls of other dicotyledons and of gymnosperms as reported in the literature.

While much information is now available on the composition and structure of plant cell walls (3, 6, 7, 36), we have very little data on the biochemical changes occurring in the cell wall of a single higher plant cell during the process of cell elongation and maturation. The most extensive studies to date on the structure of the primary cell wall of plant cells have been carried out in Albersheim's laboratory (2, 3, 8, 15, 22, 35, 50, 56). With one exception (35), these studies have been performed exclusively with plant cells grown in culture. Although such cells possess the distinct advantage of representing a relatively homogeneous cell type, they are not well suited for physiological studies which could correlate biochemical changes in the wall with cell elongation and maturation. Nevertheless, two important concepts have evolved from this work: (*a*) that the primary cell wall is composed of only a limited number of distinct polymers; and (*b*) that the primary cell walls of cultured dicotyledonous cells, while quite similar to each other, are distinctly different from those of the cell walls derived from the monocotyledonous cell cultures which were examined (15). Although in earlier work (35), the elongating cell wall was referred to as a dynamic structure, implicit in all of this group's later work has been the concept that the primary cell wall is a relatively constant structure, and "the average chemical composition of the wall is not altered" (2) during wall growth.

Some attempts have been made to determine changes in cell wall composition associated with the process of cell elongation, but these studies have almost invariably utilized tissues consisting of various cell types. Labavitch and Ray (26, 27) have recently shown that auxin treatment of pea epicotyls enhances the solubility of wall xyloglucan, and that this change may be correlated with growth. Increases in the level of the hydroxyproline-rich wall protein (expressed as per cent of the fresh weight of tissue) toward the end of elongation have been observed, and the suggestion has been made that this component serves to stabilize the wall from further extension (16, 42). However, recent data of Klis (25) indicate that when hydroxyproline content is expressed as per cent of the dry weight of cell wall, its content continually increases during cell elongation, but that there is no further increase once the cells have obtained their final length. Also of interest are studies showing that a specific decrease in noncellulosic glucose, which is accompanied by a roughly compensating increase in cellulose, occurs in the cell wall of *Avena* coleoptiles elongating in response to auxin in the absence of an external carbon source (31, 39), and it has been suggested that changes in noncellulosic wall glucan may be involved in wall loosening (34).

The developing cotton fiber represents a good experimental system for studying biochemical changes associated with cell elongation and/or maturation. Cotton fibers are single cells which elongate synchronously over a period of almost 3 weeks. Sufficient material can be readily obtained for biochemical analyses of the cell wall. Further potential advantages are that isolated unfertilized ovules with their associated fibers can be

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cultured *in vitro* in a synthetic medium supplied with IAA and GA_3 (10). Thus, the cell wall compositions of cell walls derived from cells growing on the plant can be compared with those of fibers grown in culture. It also seems (37, 41, 54 and Delmer, unpublished results) that the cotton fiber deposits cell wall material throughout its entire length during growth in a manner similar to that of most other plant cells (40, 45). It is still uncertain whether elongation proceeds primarily by tip growth or over-all elongation, although recent unpublished results from our laboratory strongly suggest the latter type of growth. Because the cell wall composition of the elongating fiber can be easily expressed as g/unit cell wall length, it is possible to examine absolute rather than relative changes in composition occurring during primary cell wall elongation and maturation.

In view of the commercial importance of the cotton fiber, it is surprising that except for studies on cellulose, very little is known about the composition of the primary wall of the cotton fiber. The most distinctive feature of the cotton fiber is that the secondary wall is pure cellulose. As shown in the careful studies by Marx-Figini (32, 33), the degree of polymerization of primary wall cellulose is low and heterogenous (DP from 2,000–6,000) while the onset of secondary wall deposition, which occurs toward the end of elongation, is characterized by an abrupt change in degree of polymerization, with the newly deposited secondary wall cellulose having a high and remarkably homogeneous degree of polymerization (DP = 14,000).

The purpose of the work presented here has been 2-fold: (*a*) to analyze the composition of the cell wall of the developing cotton fiber throughout the course of elongation and secondary wall formation; and (*b*) to compare the composition of walls of fibers grown in the plant with those developing on ovules cultured *in vitro*. The results indicate that the cell wall of the cotton fiber is a dynamic structure, the composition of which is continually changing throughout development, and that the cell walls of cultured fibers are quite similar to those of plant-grown fibers. In addition, compositional and linkage analyses indicate that the primary cell wall of the cotton fiber is similar to the reported compositions of primary cell walls from cultured cells of a gymnosperm and a variety of dicotyledonous plants.

MATERIALS AND METHODS

Growth. Seeds of *Gossypium hirsutum* L. ('Acala SJ-1') were obtained from Hubert Cooper, Jr., USDA/ARS U.S. Cotton Research Station, Shafter, Calif. They were germinated and grown as described by Beasley and Ting (9) with the exception that plants were maintained in growth chambers under the following light and temperature regime: 11.5 hr light (10 hr, incandescent and fluorescent lamps, followed by 1.5 hr from incandescent lamps only) at 33 C; 12.5 hr dark at 22 C. Plantings were made at 3-week intervals and the plants were discarded after 4 months. On the morning of anthesis, flowers were labeled with tags and were fertilized by gently brushing the anthers to dislodge pollen. At any given time, no more than three bolls were allowed to develop on any one plant. Fiber lengths were measured as described by Schubert *et al.* (44).

Ovule Culture. Unfertilized ovules were taken on the day of anthesis and cultured as described by Beasley and Ting (9, 10). Their basal medium was supplemented with 5 μM IAA and 0.5 μM GA_3. The ovules were incubated in darkness at 34 C.

Cell Wall Preparation. Fibers, either plant- or culture-grown, of a known age were quantitatively removed from the ovule with forceps, immediately immersed in liquid N_2, lyophilized, and then finely ground, with dry ice to facilitate grinding, in a mortar and pestle. Cell walls were prepared from the fiber preparation essentially by the method of Talmadge *et al.* (50). The lyophilized fibers were thoroughly homogenized in 10 volumes (v/w) of cold 100 mM K-phosphate (pH 7), centrifuged in a clinical centrifuge for 15 min, and the supernatant discarded. In this manner, the pellet was successively washed at least three times with 10 volumes of each of the following: 500 mM K-phosphate buffer (pH 7), distilled H_2O, 1:1 chloroform-methanol and, finally, acetone. The resultant residue was frozen, lyophilized, and following drying to constant weight, the cotton fiber cell wall preparations were stored over phosphorus pentoxide at room temperature in a vacuum desiccator. Cell walls of cultured sycamore maple cells, prepared by the procedure of Talmadge *et al.* (50), were the gift of D. Bauer and L. Cardemil de Balboa.

Extracellular Medium. Twenty-five ml of cell-free culture medium were lyophilized, resuspended in 20 ml of 70% ethanol, and stored 7 days at 4 C, at which time the supernatant was carefully decanted. The remaining precipitate was resuspended in 70% ethanol, stored at 4 C, and centrifuged at 3000*g* for 20 min; the resulting pellet was lyophilized and analyzed for neutral sugar composition.

Analyses for Starch. Cell walls of a variety of ages were tested for the presence of starch by the iodine reaction (52). At no age was a positive reaction observed. Cell wall preparations were not treated with α-amylase prior to further analyses.

Urea Extraction of Walls. Three mg of cell wall were suspended in 4 ml of a solution as described by Allen and Neuberger (5) containing 8 M urea, 2% SDS, and 2.5% mercaptoethanol. The suspension was heated at 100 C for 5 min, then cooled, and centrifuged in a clinical centrifuge for 10 min. The supernatant was decanted and saved for subsequent analyses. The pellet was thoroughly washed with 70% ethanol and analyzed for amino acid content.

Preparation of Cotton Fiber Cellulose. Cell walls were freed of noncellulosic, organic constituents by digestion for varying lengths of time with an acetic/nitric reagent according to Updegraff (51).

Endopolygalacturonase Treatment. Cell walls (from 16 DPA, plant-grown fibers) were digested (by M. McNeil in the laboratory of P. Albersheim) as described by Talmadge *et al.* (50) using endopolygalacturonase purified from *Colletotrichum lindemuthianum*.

Chemical Determinations. The cellulose content of the cell walls was determined by the method described by Updegraff (51). Uronic acid concentrations were determined by the method of Blumenkrantz and Asboe-Hansen (13). Determinations of uronic acids were performed either directly on wall preparations or using 2 N TFA[3] hydrolysates (121 C, 75 min) of the cell walls. Within experimental error, the two methods gave comparable results. Total neutral sugars were assayed using similar 2 N TFA hydrolysates by the method of Park and Johnson (38).

Ash content and total N (Kjeldahl procedure) were analyzed by Spang Micro-Analytical Laboratories, Ann Arbor, Mich. Amino acid analyses showed that the average mol wt of peptide-bound amino acid in the wall at all ages was approximately 112 and, under the assumption that all N in the wall is present in amino acids, per cent by weight protein in the cell wall was calculated accordingly. Amino acid analyses were performed using a modified Technicon Autoanalyzer as described by Lamport (29). The hydrolysates were prepared using 3 mg of cell wall which were added to microvial with redistilled constant boiling HCl. Hydroxyproline content was analyzed separately by a modification of the method of Kivirikko (24) as described by Lamport and Miller (30).

SDS mol wt determinations were performed by the method of Weber and Osborn (53) as described by Heages (20). Purified vicilin, a storage protein isolated form *Phaseolus aureus* having subunit mol wt of 63,000, 50,000, 29,500, and 24,000, was used as a standard for mol wt.

[3] Abbreviations: TFA: trifluoroacetic acid; DPA: days postanthesis.

Neutral Sugar Analyses by Gas Chromatography of Alditol Acetates. Alditol acetates of neutral sugars were prepared essentially by the method of Albersheim *et al.* (4). Gas chromatographic separations, when not combined with MS, were carried out on a Varian 2100 gas chromatograph with helium as carrier gas. Glass columns (180 cm × 0.2 cm i.d.) were packed with a mixture of 0.2% poly(ethylene glycol adipate), 0.2 % poly(ethylene glycol succinate), and 0.4% silicone XE-1150 on Gas-chrom Q (80–100 mesh). Chromatography was performed with temperature programing at 2 C/min from 130 to 180 C with a helium flow rate of 35 ml/min. Gas chromatographic peaks were integrated with a Hewlett-Packard 3370 A integrator, equipped with an internal timer. The peak areas and retention times were expressed relative to the internal standard of inositol added to each sample prior to TFA hydrolysis. Reagent grade standard sugars, obtained commercially, were used without further purification. Standard sugar mixtures were run with each set of determinations.

Methylation Analyses. Methylation of unfractionated cell walls was carried out by the method of Hakomori (19) as described by Sandford and Conrad (43). Following methylation, 1 ml of chloroform-methanol (1:1, v/v) was added and this solution dialyzed for 2 days against four changes of 2 liters each of distilled H_2O. The dialysate was evaporated under N_2 at 30 C, resuspended in chloroform-methanol (1:1, v/v), and reevaporated. The dry residue was then extracted with chloroform and the material solubilized was brought to dryness under N_2 at 30 C and hydrolyzed for 1 hr at 121 C with 0.5 ml of 2 N TFA containing internal inositol standard. The resulting partially methylated aldoses were reduced to their corresponding alditols with 0.5 ml of 0.4% sodium borodeuteride (rather than sodium borohydride) in 3 N NH_4OH to aid in identification of ambiguous mass spectral fragments. The presence of deuterium in the anomeric carbon provides a distinguishable difference between the fragmentation patterns derived from stereochemically symmetrical derivatives. These partially methylated alditols were then converted to their corresponding alditol acetates (4). Gas chromatographic separations of the acetylated derivatives were carried out as described for neutral sugar analyses except that gas chromatography was performed with temperature programming at 1 degree/min from 110 to 190 C with a helium flow rate of 30 ml/min. When combined with MS, separations were performed with the same column packing on an LKB 9000 combined GLC-mass spectrometer capable of repetitive scanning as described by Laine *et al.* (28). A computer data system with a PDP-8/E minicomputer was used as described by Sweeley *et al.* (49) and modified by Laine *et al.* (28). Derivatized maltose, laminaribiose, and sycamore maple cell walls were used as a source of standards for relative retention times. Final linkage designations were assigned using a combination of our own data as well as published values on retention times and mass spectral data for derivatized sugars.

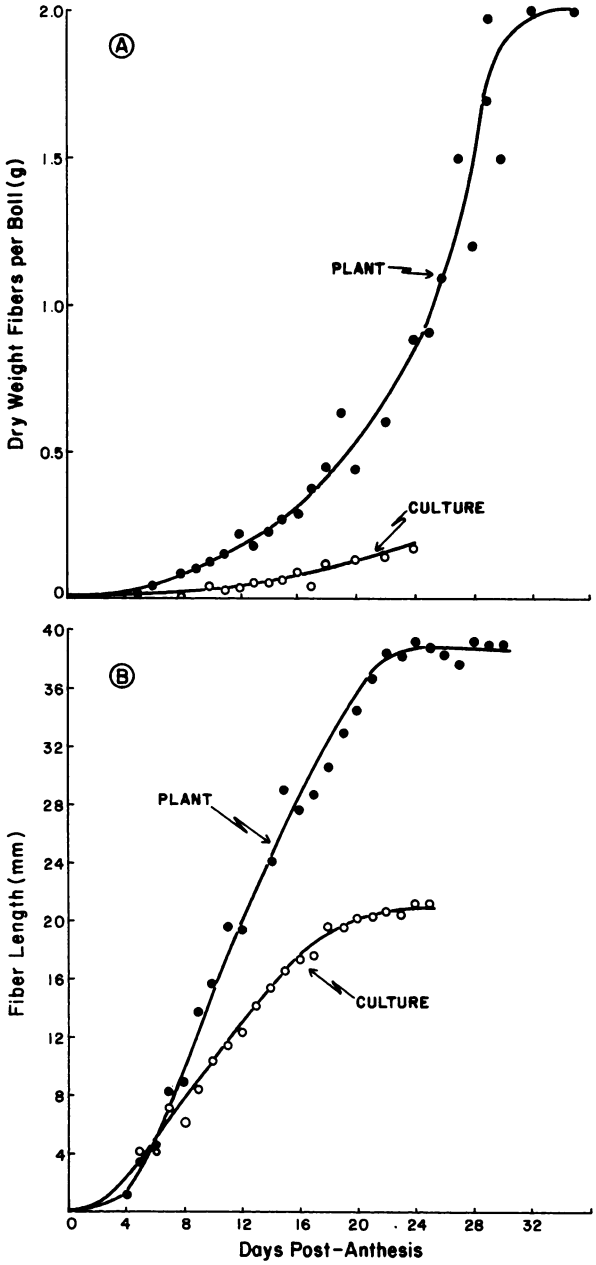

FIG. 1. Basic parameters of fiber development. A: changes in dry weight during development. The dry weight of total fibers harvested is expressed per boll in the case of plant-grown fibers, and in the case of culture-grown fibers is expressed per 24 ovules. B: fiber length as function of time.

RESULTS

Growth and Development of the Boll and Fiber. Although it is well documented that cotton fibers develop synchronously in the boll (44), it was necessary to establish this for our growth conditions. For this purpose, we measured boll length, boll weight, fiber length, and dry weight of fibers per boll as a function of time postanthesis. The courses of increase in boll length and weight were found to be highly reproducible. Thus, these two easily measured parameters can be used in judging whether or not a boll has developed normally and will yield cotton fibers characteristic of the physiological age (data not shown). The dry weight of fibers/boll (per 24 ovules in the case of culture-grown fibers) is shown in Figure 1A. For plant-grown fibers, an increase in dry weight of fibers/boll is observed until about 30 DPA, after which there is no further increase. The dry weight of culture-grown fibers also undergoes an increase with development although at a much slower rate. In order to determine the rate of cell elongation, fiber lengths at various stages of development were measured (Fig. 1B). Both the rate of elongation and the final length of the fibers grown in culture are about half that of fibers grown on the plant. Since the dry weight/boll equivalent of cultured fibers is considerably less than half that of plant-grown fibers even though the final length is reduced by only half, fiber number appears to be reduced in culture. The cessation of elongation also occurs earlier in culture; for plant-grown fibers, elongation sharply declines at 22 DPA, compared to about 18 DPA in culture. The maximal rate of fiber elongation occurs between 6 and 12 DPA for plant-grown fibers.

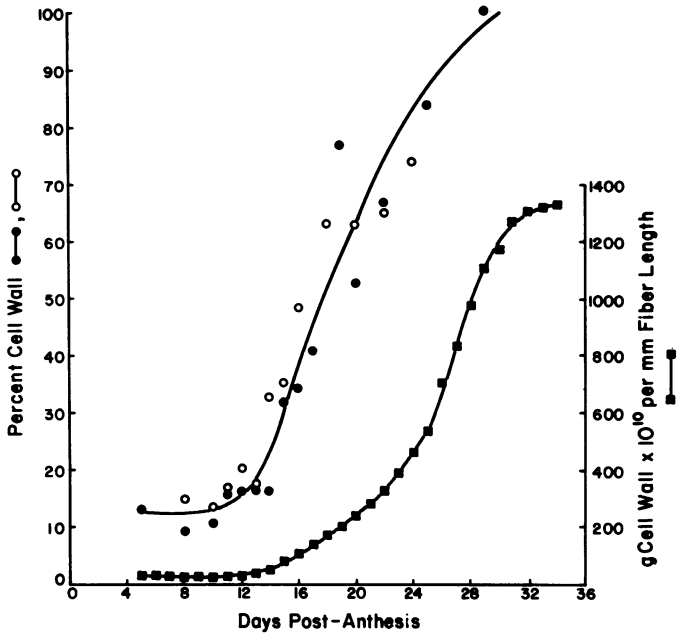

FIG. 2. Cell wall yields during fiber development. Per cent cell wall is defined as the per cent of the total dry weight of the fiber cells which is recovered as cell wall material. (●): plant grown fiber; (○): cultured fibers. The procedure for the calculation of g/mm of cell wall (■) is given in the text; data for this parameter are calculated only for plant-grown fibers.

Cell Wall. Cell walls were prepared from lyophilized fibers and the per cent of the total dry weight of the fiber cell recovered as cell wall material was calculated for fibers of various ages. Cell wall percentages from both plant- and culture-grown fibers (Fig. 2) increase at similar rates with the developmental age of the fiber. The lower fiber production in culture-grown fibers has not affected the rate at which cell wall material is being deposited/weight of fiber under these conditions.

In order to measure the quantity of cell wall/unit length of fiber (plant-grown), the following calculation was made:

$$\frac{\text{g cell wall}}{\text{mm fiber length}} = \frac{\text{g cell wall}}{\text{g fibers}} \times \frac{\text{g fiber}}{\text{boll}} \times \frac{\text{boll}}{\text{no. of fibers}} \times \frac{1}{\text{mm fiber length}}$$

This calculation assumes that the number of fibers/boll is constant throughout development and equals 384,000 lint fibers[4] (C. A. Beasley, personal communication). The g cell wall/mm fiber length is relatively constant during the early phase of cell wall elongation (Fig. 2). The "thickness" of the wall (g/length) begins to increase at 12 DPA, several days prior to the onset of secondary wall cellulose synthesis.

Cellulose. The content of cellulose, expressed as a weight per cent of the wall material analyzed, is given in Figure 3A for both plant- and culture-grown fibers. The timing of what has been considered to be the changeover from primary to secondary wall synthesis (44) is very exact in the plant-grown fibers, as evidenced by the abrupt increase in cellulose content between 16 and 18 DPA, and the distinct appearance of birefringence in the fibers observed by polarized light microscopy between days 18

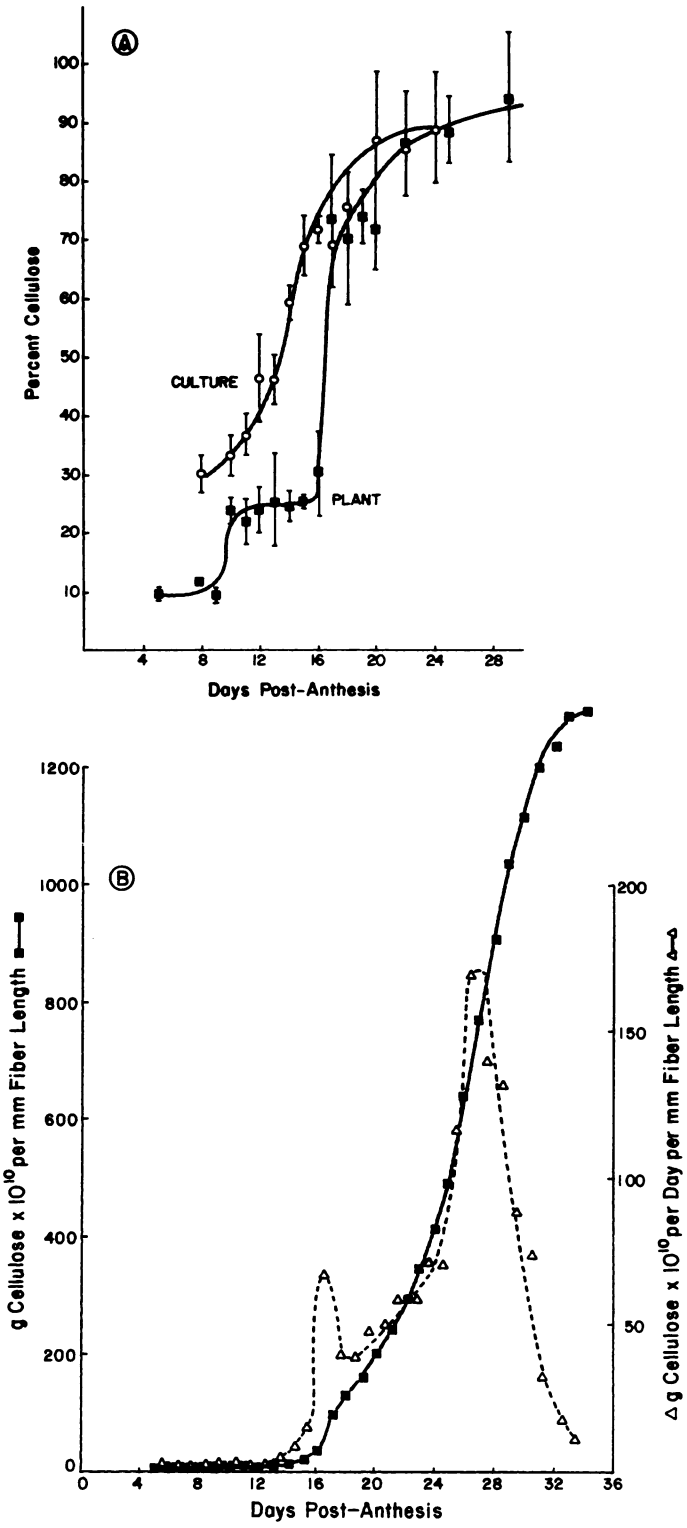

FIG. 3. Changes in cellulose content of the cell wall during fiber development. A: per cent of the weight of the cell wall that is cellulose is given for walls derived from both plant- and culture-grown fibers. Standard deviations are given for multiple samples, representing multiple assays and in most cases multiple fiber harvests and multiple cell wall preparations. B: solid line represents the g of cellulose/mm fiber length and was calculated by multiplying the g/unit length of total cell wall at a particular fiber age by the per cent by weight of cellulose at that age taken from the curves in A. The calculation for the apparent rate of cellulose deposition is given in the text.

[4] It should be noted that throughout this paper we have ignored any possible contribution of fuzz fibers to our analyses. In Acala SJ-1, the per cent by dry weight of mature fiber yield which is accounted for by these fuzz fibers is <15% (C. A. Beasley, personal communication). The calculations are also made with the assumption that wall material is deposited uniformly throughout the fiber length.

and 20. Between 16 and 17 DPA, the per cent of the wall that is cellulose approximately doubles in 1 day. In culture-grown fibers this transition occurs earlier and extends over a longer period of time. In plant-grown fibers, there appear to be three distinct phases of cellulose deposition; two phases occur within the elongation phase when the primary wall is synthesized, and the third phase occurs near the end of elongation when secondary wall synthesis takes place. It was surprising to find two apparent phases of cellulose deposition during primary wall synthesis. However, percentage changes in cellulose content are not necessarily an indication of the rate at which cellulose is being deposited. Converting the per cent of the wall represented by cellulose to g cellulose/mm fiber length more clearly illustrates the changes in cellulose content occurring in an elongating cotton fiber (Fig. 3B). Such a plot shows that the bulk of cellulose deposition actually occurs many days later than would be indicated from the plot of per cent cellulose *versus* time. To understand this, it must be realized that at 16 DPA the cell wall is still relatively thin and small increases in cellulose deposition at this time will cause large changes in the cellulose content expressed as per cent of the total wall, while as the cell wall begins to thicken with secondary wall cellulose, the percentage changes become smaller, but absolute deposition increases greatly.

This phenomenon is even more clearly illustrated by computing the apparent rate of cellulose deposition/unit length of fiber. This computation was done by assuming no turnover of cellulose and calculating the changes in content/length of fiber/day with corrections for increases in fiber length as follows:

$$\text{rate} = \frac{(G_n \times L_n) - (G_{n-1} \times L_{n-1})}{L_{avg}}$$

where G_n equals the g cellulose/mm fiber length at day n, L_n equals the mm fiber length at day n, and

$$L_{avg} = \frac{L_n + L_{n-1}}{2}$$

Plotting the data in this manner shows that the rate of deposition is fairly constant throughout most of primary cell wall elongation. At 14 DPA it begins to increase and continues to do so through 16 DPA. Between 16 and 17 DPA there is an abrupt 4-fold increase in the rate of cellulose deposition. Surprisingly, the rate of cellulose deposition reproducibly declines before undergoing a second, even greater increase. This second rise coincides with the sharp decline in the rate of fiber elongation (compare Fig. 1). The maximal rate of cellulose deposition occurs between 26 and 28 DPA after which the rate drops sharply.

Neutral Sugars. Purified cell walls from both plant- and culture-grown fibers of various ages were hydrolyzed with 2 N TFA and the neutral sugars determined as their alditol acetates. The neutral sugars obtained from cell walls by this procedure include rhamnose, fucose, arabinose, xylose, mannose, galactose, and glucose (noncellulosic). The quantity of each of the monosaccharides released from the wall upon hydrolysis is expressed as a mole per cent of the total amount of neutral sugars detected (Table I). The total weight of the wall which could be accounted for by the sugars released in the TFA hydrolysis treatment decreased from 32% at 5 DPA to 6% at 29 DPA. The results of assaying the TFA hydrolysate supernatant for total reducing sugars by a colorimetric assay are also presented and, for most ages, are in agreement with the results obtained by GLC. We concluded from these data that the relative amounts of the individual sugars present in the walls of plant-grown fibers are variable with age, and that similar relative changes are observed in cell walls derived from plant- and from culture-grown fibers. The sugars that undergo the greatest changes when expressed as a weight per cent are arabinose and noncellulosic glucose (Fig. 4A). The changes in content of these neutral sugars from cell walls of plant-grown fibers, expressed as g of neutral sugar/unit length of fiber are shown in Figure 4B. These data also show that as an absolute amount of the total wall, the noncellulosic glucose content undergoes a large increase during development which

TABLE I

Neutral Sugar Composition of the Cell Walls of Plant- and Culture-grown Fibers of Various Ages

Purified cell walls were hydrolyzed and the neutral sugars were determined by GLC of the alditol acetate derivatives as described in Materials and Methods. Each sugar is expressed as a mole percent of the total neutral sugars detected. Each value represents an average of at least 4 alditol acetate preparations, and in many cases, of multiple cell wall preparations from multiple harvests. Standard deviations are given as superscripts. The total neutral sugar content was determined independently by a colorimetric assay (38) as well as by GLC.

		Age of Fibers (DPA)												
Sugar		5	8	10	12	14	15	16	18	20	22	24	25	29
Plant-Grown Fibers														
Rhamnose		$5.9^{0.6}$	$7.9^{0.4}$	$7.4^{0.9}$	$8.5^{0.8}$	$8.4^{0.5}$	$6.7^{0.4}$	$4.3^{0.2}$	$5.1^{0.0}$	---	$2.3^{0.2}$	---	3.1	$1.7^{0.3}$
Fucose		$.9^{0.3}$	$1.2^{0.1}$	$1.2^{0.7}$	$1.4^{0.2}$	$1.2^{0.3}$	$1.0^{0.7}$	$0.4^{0.4}$	$0.5^{0.6}$	---	$0.4^{0.1}$	---	0.2	$0.3^{0.0}$
Arabinose		$24.4^{2.6}$	$31.8^{3.1}$	$32.8^{4.6}$	$22.3^{2.6}$	$22.3^{2.1}$	$13.6^{0.2}$	$7.9^{0.6}$	$8.6^{1.3}$	---	$4.1^{0.1}$	---	5.6	$5.5^{0.2}$
Xylose		$7.7^{0.5}$	$10.0^{0.4}$	$10.2^{1.4}$	$10.8^{1.2}$	$10.3^{0.4}$	$9.0^{0.3}$	$6.6^{0.4}$	$8.5^{0.6}$	---	$4.0^{1.0}$	---	3.7	$3.5^{0.1}$
Mannose		$3.5^{0.8}$	$3.9^{0.1}$	$3.9^{0.3}$	$4.2^{0.3}$	$3.9^{0.3}$	$3.6^{0.6}$	$1.7^{0.5}$	$1.9^{0.6}$	---	$2.5^{1.6}$	---	1.3	$1.2^{0.1}$
Galactose		$12.9^{0.7}$	$15.8^{0.2}$	$17.1^{1.9}$	$16.8^{0.7}$	$17.8^{0.3}$	$13.4^{0.7}$	$7.4^{0.8}$	$8.4^{0.0}$	---	$4.5^{1.2}$	---	5.9	$4.9^{0.1}$
Glucose		$45.0^{2.0}$	$29.5^{3.6}$	$28.0^{6.3}$	$36.2^{1.6}$	$36.2^{2.5}$	$52.7^{0.7}$	$71.7^{0.9}$	$67.1^{0.9}$	---	$82.4^{3.6}$	---	80.4	$83.0^{0.0}$
Total Percent of Cell Wall	GLC:	$32.4^{2.3}$	$25.0^{4.0}$	$23.2^{2.1}$	$21.7^{4.4}$	$21.0^{2.2}$	$13.8^{2.3}$	$13.8^{3.5}$	$10.0^{1.1}$	---	$10.1^{0.1}$	---	10.0	$5.8^{0.3}$
	Color:	---	---	25.0	---	24.0	21.0	21.0	11.0	---	---	---	---	8.0
Culture-Grown Fibers														
Rhamnose		---	$8.6^{0.6}$	$9.8^{1.1}$	$8.7^{0.8}$	$6.1^{0.9}$	---	$4.6^{0.8}$	$3.0^{0.5}$	$2.8^{0.4}$	---	$2.7^{0.1}$	---	---
Fucose		---	$1.1^{0.1}$	$1.1^{0.2}$	$1.2^{0.4}$	$0.7^{0.1}$	---	$0.5^{0.2}$	$0.1^{0.1}$	$0.2^{0.1}$	---	$0.2^{0.1}$	---	---
Arabinose		---	$29.2^{1.2}$	$29.6^{2.6}$	$26.4^{0.3}$	$14.9^{2.2}$	---	$12.2^{1.0}$	$8.0^{1.9}$	$8.2^{2.2}$	---	$7.2^{1.9}$	---	---
Xylose		---	$12.3^{1.4}$	$17.4^{1.3}$	$16.7^{1.0}$	$17.5^{1.9}$	---	$12.5^{1.2}$	$16.2^{0.8}$	$9.1^{0.6}$	---	$9.7^{0.5}$	---	---
Mannose		---	$3.6^{0.1}$	$3.5^{0.4}$	$3.0^{0.5}$	$2.0^{0.5}$	---	$1.3^{0.3}$	$1.2^{0.3}$	$1.1^{0.5}$	---	$1.9^{0.6}$	---	---
Galactose		---	$18.6^{0.4}$	$17.8^{0.8}$	$15.9^{0.5}$	$9.2^{0.9}$	---	$7.8^{0.9}$	$4.3^{0.6}$	$4.7^{1.1}$	---	$5.0^{0.3}$	---	---
Glucose		---	$26.9^{0.9}$	$20.6^{2.5}$	$27.9^{2.6}$	$49.8^{5.3}$	---	$61.1^{3.5}$	$67.2^{2.1}$	$73.9^{4.6}$	---	$73.3^{1.8}$	---	---
Total Percent of Cell Wall	GLC:	---	$27.3^{3.3}$	$23.3^{4.2}$	$21.1^{1.4}$	$17.3^{4.0}$	---	$15.7^{2.1}$	$16.4^{3.0}$	$12.3^{0.7}$	---	$11.6^{4.1}$	---	---

essentially parallels the rise in total neutral sugars. An approximate 2-fold rise in the content of xylose is observed throughout development, and a 2-fold decrease in arabinose. The other neutral sugars show no significant change in content.

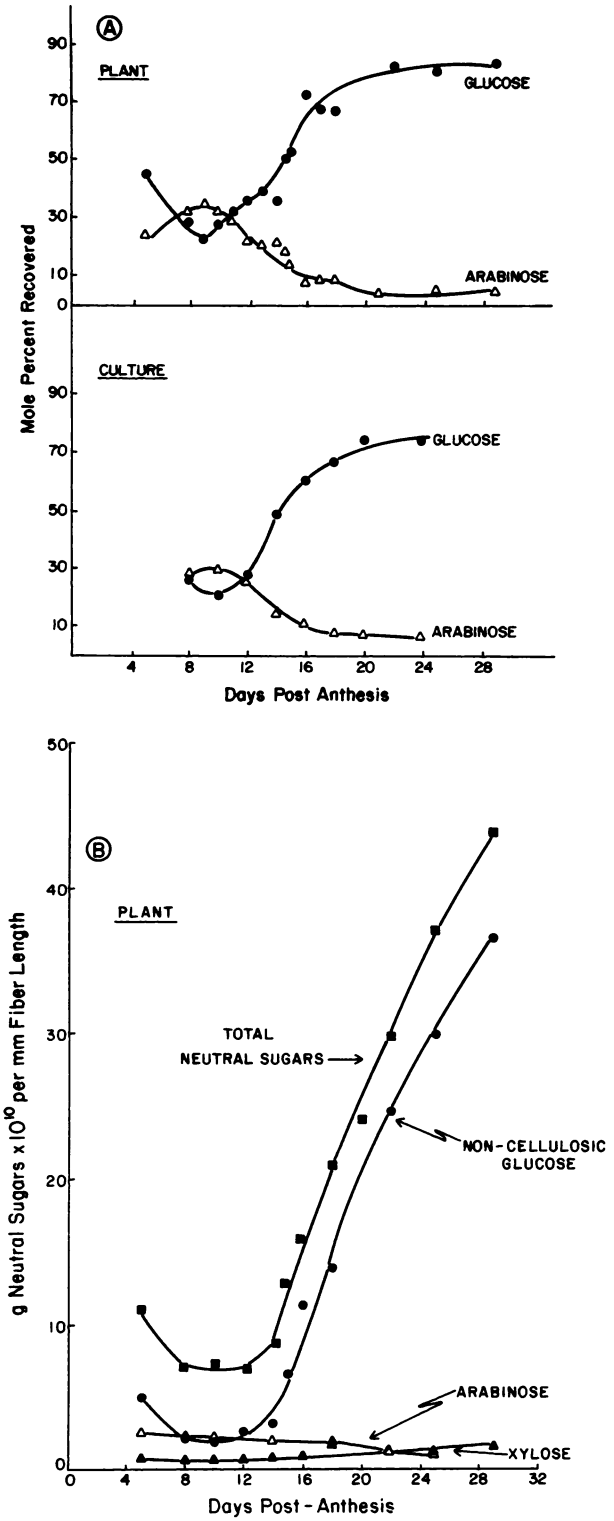

FIG. 4. Changes in neutral sugar content of cell walls during fiber development. A: changes in mole per cent of arabinose and noncellulosic glucose in cell walls from plant and culture grown fibers. B: changes in g/mm fiber length of total neutral sugars, noncellulosic glucose, arabinose, and xylose during development of cell walls derived from plant-grown fibers. The values were calculated by multiplication of the g/mm fiber length of total cell wall at any particular age by the per cent by weight of the sugar at that age.

Extracellular Polysaccharides. The ethanol-precipitable fraction obtained from cotton ovule culture medium was analyzed for neutral sugar composition at five different developmental time points. The sugars detected include arabinose, xylose, galactose, and glucose. An increase in the per cent of arabinose, xylose, and galactose, and a decrease in the amount of glucose present were observed during the time course of development examined. These data indicate that culture-grown fibers may secrete polysaccharides. This observation has not been pursued but may prove useful for future studies on wall structure.

Residual Sugars Associated with Cellulose. It is generally accepted (50) that cellulose is resistant to hydrolysis by TFA (2 N, 121 C, 1-2 hr). Because we observed a large rise in noncellulosic glucose occurring nearly coincident with the onset of secondary wall cellulose deposition, it was necessary to reconfirm that cellulose was indeed resistant to our TFA hydrolysis conditions in order to be certain that the rise in noncellulosic glucose was not really an artifact due to partial hydrolysis of cellulosic glucose. In an effort to quantitate the susceptibility of cellulose to hydrolysis by 2 N TFA, it was found that small, but significant, quantities of sugars other than glucose (arabinose, xylose, mannose) were released during hydrolysis both from our own preparations of cotton fiber cellulose and from thoroughly washed α-cellulose (Sigma Chemical Co.). The total amount of sugars released (including glucose) after a series of sequential hydrolyses in TFA never exceed about 11% of the original weight of the cellulose. The glucose released after sequential hydrolyses represents less than 3% of the weight of the cellulose prepared from cotton fibers, indicating that more than 97% of cellulose treated in this way is indeed resistant to 2 N TFA hydrolysis.

In a similar study, we found that the predominant residual sugars associated with the cellulosic fraction of sycamore maple cell walls (obtained by an acetic/nitric digest of total cell walls) were xylose, glucose, and to a lesser extent, mannose. This result indicates a comparable interrelationship between the structural components of these cells walls with that of cotton fibers cell walls, in that these same three sugars are released in both cases. Adams and Bishop (1) have also reported the presence of residual sugars associated with cotton fiber-cellulose. It would be of interest to characterize further this residual fraction by methylation studies. One possibility is that the residual xylose and glucose represent a residual fraction of a xyloglucan polymer such as that proposed to be in close association by hydrogen bonding with the cellulose microfibrils in sycamore cell walls (8). Another possibility is that the xylose, glucose, and mannose are present as trace amounts of xylan and glucomannan, constituents normally associated with secondary walls (7).

Uronic Acids. The uronic acid component is difficult to quantitate owing to the resistance of the aldobiuronic acid glycosidic linkage to acid hydrolysis (55). These compounds can be partially degraded before they are hydrolyzed, and substantial losses may thus occur. The data on the quantitation of total uronic acids may represent an underestimate, but they at least provide an indication of variation in uronic acid content during fiber development. Figure 5 shows the variation in uronic acid content expressed both as a per cent by weight of the wall and as g/unit fiber length. Expressed in the latter way, there is a steady increase in uronic acid content until 16 DPA, and an abrupt decline thereafter. The sharp decline in uronic acid content seems to represent actual removal from the wall since, even if synthesis of this component ceases at 16 DPA, dilution by elongation cannot account for the total decrease in uronic acid

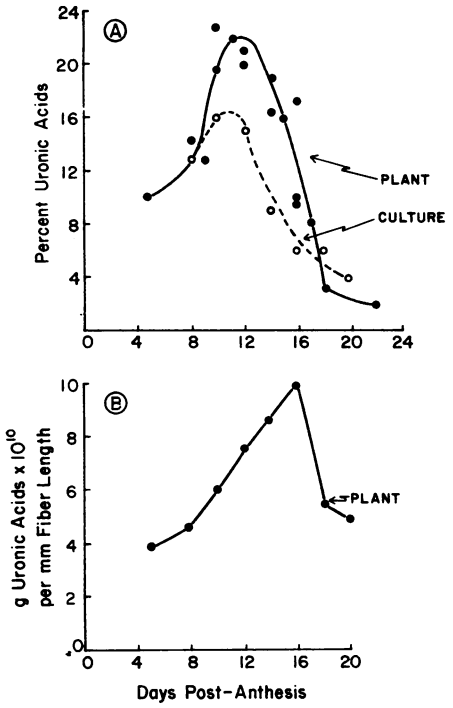

FIG. 5. Uronic acid content in the fiber wall during development. A: per cent by weight uronic acids is given for both plant- and culture-grown fibers. Each data point is an average of triplicate determinations on a wall preparation. Where two points are given for the same age, they were obtained by independent triplicate determinations. (●): plant-grown fiber cell walls; (○): culture-grown fiber cell walls. B: uronic acid content expressed as g/mm fiber length. Data points were calculated by multiplication of the g/mm fiber length of total cell wall at any particular age by the per cent by weight uronic acids at that age taken from the line drawn in (A) for plant-grown fibers.

content between 16 and 18 DPA. This can be seen from the fact that the absolute content drops by about 50% between 16 and 18 DPA whereas the fibers only increase in length by about 13%. Another possibility, which is difficult to rule out, is that the uronic acids become increasingly difficult to detect by this assay procedure as the fiber wall changes composition during this stage of development. The bulk of the uronic acids are probably present as polyuronides; this is indicated by a preliminary experiment which has shown that 60 to 70% of the total uronic acids of the wall can be solubilized by digestion of cell walls with *Colletotrichum* endopolygalacturonase.

Protein. Table II shows the amino acid composition of the cell walls from plant- and culture-grown fibers as an average of all of the data for the various ages examined. The data for each age are not presented since no significant change in composition with fiber age was found. This can be seen from the low standard deviations obtained when all ages are considered together. Approximately 50% of the amino acids are hydrophobic, and at any age the composition shows no preponderance of any particular amino acid. No differences are observed in amino acid composition for cell walls isolated from plant- and culture-grown fibers. The results of a separate assay for hydroxyproline are also shown in Table II. This amino acid comprises less than 0.6% of the weight of the wall at any age. Because these values are so low and were repeated only once, we have not attempted to calculate changes, if any, in the absoulte content of this amino acid during development.

The total protein content, measured by analyses for total N and calculated on the assumption that all N is present as peptide-bound amino acids, is surprisingly high when expressed as weight per cent of the wall, especially at the youngest age examined, but declines noticeably later in development (Fig. 6). However, when expressed as g/unit length of fiber, the content rises sharply between 12 and 16 DPA, and subsequently declines. As for uronic acids, calculations show that this decrease in protein content indicates an absolute removal from the wall during this later period.

In an attempt to ascertain whether or not the cell wall protein is a true structural component of the wall, or whether at least a portion of it could have been adsorbed from the cytoplasm during cell wall preparation, 14 DPA walls were extracted with a solution of SDS, urea, and mercaptoethanol for 5 min at 100 C and the residue was analyzed for residual protein. This treatment should remove any protein not covalently bound to cell wall polysaccharides. More than half of the protein of the walls was indeed solubilized by this treatment, but the amino acid composition of the protein remaining bound to the wall remained quite similar to that of unextracted walls.

The supernatant of the SDS-urea-mercaptoethanol extraction was dialyzed and analyzed by SDS gel electrophoresis to see to what extent, if any, polypeptides had been released from the wall. The results (not illustrated) clearly showed at least eight

TABLE II

Comparison of Amino Acid Composition of Cell Wall Protein for Cell Walls Derived from Plant and Culture Grown Fibers.

Each value represents an average of the mole per cent of each amino acid detected at a variety of ages for both plant and culture grown fibers. For plant-grown fibers, data were pooled from duplicate samples obtained from fibers of 5, 9, 10, 11, 12, 14, 15, 16, 17, and 29 DPA; for culture-grown, from samples of fibers of 10, 12, 14 and 16 DPA. Standard deviations are given.

Amino acid	Plant Average	Culture Average
ASP	10.8 ± 0.74	9.6 ± 0.33
THR	6.0 ± 0.36	5.5 ± 0.22
SER	8.4 ± 1.21	8.8 ± 0.70
GLU	11.7 ± 0.98	10.5 ± 1.5
GLY	11.8 ± 1.3	13.7 ± 1.6
ALA	10.0 ± 1.7	8.8 ± 0.73
VAL	7.0 ± 0.68	7.0 ± 0.33
MET	0.73± 0.98	0.75± 0.86
ILE	4.9 ± 0.43	4.8 ± 0.05
LEU	9.7 ± 0.58	9.5 ± 0.29
TYR	2.0 ± 0.66	1.9 ± 0.36
PHE	4.5 ± 0.27	4.4 ± 0.16
LYS	6.0 ± 1.6	8.4 ± 0.34
HIS	2.2 ± 0.56	2.5 ± 0.10
ARG	4.2 ± 0.38	2.8 ± 1.9
HYP	0.23± 0.10	0.41± 0.10

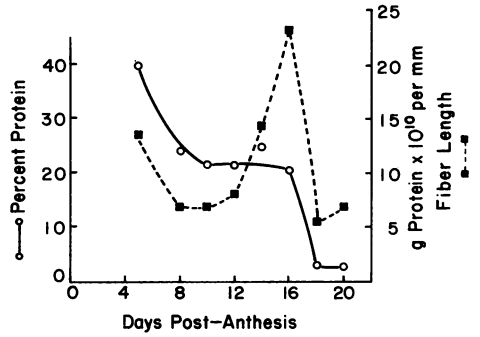

FIG. 6. Protein content in the cells wall during development of plant-grown fibers. Per cent by weight was calculated by multiplication of the per cent N by 8. Grams/unit length of fiber were calculated by multiplication of g/unit length of total cell wall at any particular age by the per cent protein at that age.

discrete bands on SDS gels, and in addition, there appeared to exist high mol wt component(s) incapable of penetrating the gel. The mol wt of the bands observed were: 15,000, 21,000, 33,000, 50,000, 63,000, 66,000, and 92,000. This supernatant was also examined for carbohydrate content, and all neutral sugars found in unextracted 14 DPA walls were also found in the urea extract, although in different proportions from those in unextracted walls. This extraction procedure resulted in preferential release of xylose and glucose and proportionally less rhamnose and arabinose. With no correction for possible losses during the procedure, the per cent of total carbohydrate released by this treatment amounts to at least 7% of the total cell wall weight. This represents approximately one-third of the total neutral sugars originally present in the cell wall, and may indicate that some covalent bonds have been broken by the procedure. Thus, information concerning how the proteins are bound to the cell wall was not clearly obtained, but may perhaps be obtained by a similar extraction at a lower temperature. Nevertheless, the data do indicate that a variety of polypeptides of discrete mol wt are present in the cell wall fraction, and that some of this protein can be removed by relatively mild treatments.

Preliminary Linkage Analyses. Permethylated cell walls from plant-grown fibers were separated into chloroform-soluble and -insoluble fractions, but only the chloroform-soluble fraction was studied further. Talmadge *et al.* (50) have shown for sycamore cell walls that the chloroform-insoluble polymers were primarily incompletely methylated cellulose and methylated oligoarabinosides attached to the hydroxyproline-rich protein. In the case of cotton fiber cell walls, it is not yet known what fraction of the wall remains insoluble under our methylation conditions. Comparison of the methylated sugar derivatives obtained with the total neutral sugar analyses indicated that the fraction analyzed here was in some respects different in composition. The presence of low amounts of mono-methyl derivatives also indicates that some undermethylated derivatives are present even in the chloroform-soluble fraction. Therefore, only limited interpretation of the results is given.

The gas chromatograms obtained for four ages of cell walls are presented in Figure 7. Tentative identifications are listed in the figure legend. Qualitatively, the derivatives obtained are similar for all ages examined (5, 8, 12, 14, 16, and 20 DPA—data shown only for 8, 12, 16, and 20 DPA). Keeping in mind the limitations stated above, several conclusions can be drawn. The first is that in view of the large number of derivatives possible, the limited number of derivatives obtained indicates that the carbohydrate polymers of the wall are of limited heterogeneity. Second, it appears that the area represented by some of the various peaks is age-dependent. Peak 5 (5-linked arabinose[5]) is prominent at all ages and represents the majority of the arabinose present, and since the total content of arabinose/unit length of fiber wall declines only slightly during development (see Fig. 4B), we have chosen to compare the relative areas of the other peaks to this peak. Using this comparison, which is of course subject to a variety of limitations as stated above, one can see that several derivatives vary in an age-dependent fashion; the most striking of these is the very large rise in the relative area of peak 9 (3-linked glucose[6]) which closely parallels the total rise in noncellulosic glucose in the cell walls (see also Fig. 4B). The area of the 3-linked glucose peak relative to the 5-linked arabinose peak is 0.007, 0.001, 0.234, 0.353, 1.406, and 1.638 at 5, 8, 12, 14, 16 and 20 DPA, respectively.

DISCUSSION

Table III summarizes the changes in the cell wall composition of cotton fibers during their development, expressed both as a per cent by weight of the wall and in terms of g/unit length of wall. A large per cent of the total weight of the wall has been accounted for by these analyses. Depending upon whether the content is expressed as a weight per cent or as g/unit length of cell wall, the interpretation of the observed changes in cell wall composition during elongation may differ greatly. Because it is more representative of actual changes in the wall, this discussion will consider only the absolute changes (g/mm fiber length). These data permit us to present an over-all picture of the changes in cell wall composition as the cotton fiber develops.

The thickness of the cell wall remains relatively constant during the early stages of cell elongation, indicating that wall synthesis is keeping pace with fiber elongation. After 12 DPA

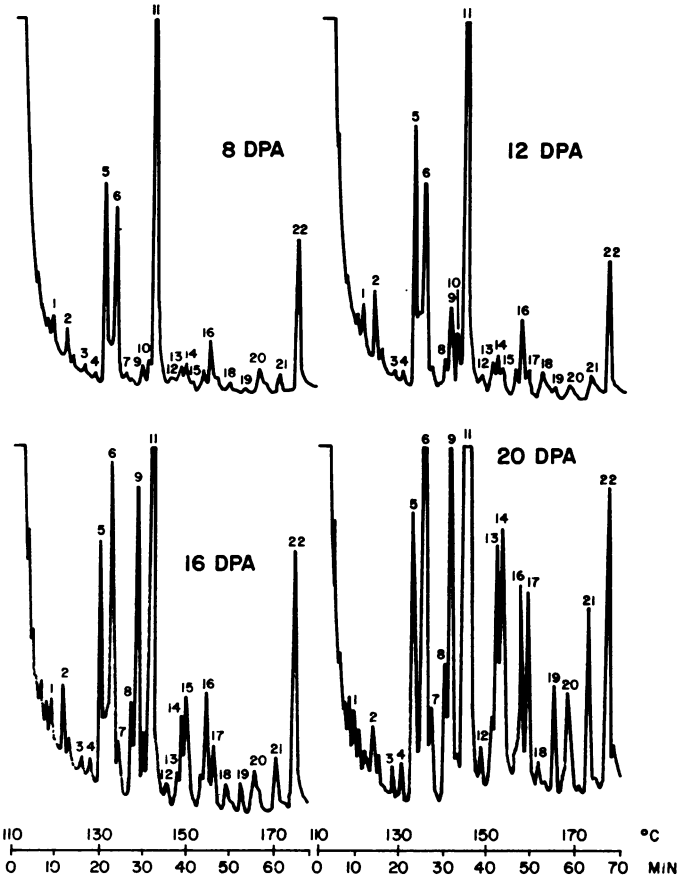

FIG. 7. Gas chromatographic separations of the alditol acetate derivatives of permethylated plant-grown cotton fiber cell wall preparations of various ages. Peak numbers refer to the following deduced derivatives: 1 = T-arabinose; 2 = T-xylose*; 3 = 2-arabinose; 4 = T-glucose; 5 = 5-arabinose*; 6 and 7 = T-galactose*, 4-xylose*, 2-xylose; 8 = 2-hexose; 9 = 3-glucose*; 10 = 3-galactose; 11 = 4-glucose*; 12 = 4- and/or 6-hexose; 13 = 6-galactose; 14 and 15 = unidentified dimethyl hexoses; 16 = 4,6-glucose*; 17 = unidentified monomethyl hexose; 18 = 3,6-galactose; 19, 20, and 21 = unidentified monomethyl hexoses. Asterisks(*) refer to sugars for which identification is supported by *both* mass spectral data and relative retention times. The other identifications are somewhat less conclusive and, in some cases, lack sufficient data for definitive identification.

[5] Data are presented by indicating the deduced linkages by which each glycosyl residue of the original sample was connected to other sugars, rather than by indicating the positions in the derivatives of the methoxy and acetyl groups. For example, 5-linked arabinose is equivalent to 1,4,5-tri-0-acetyl-2,3-di-0-methylarabinitol. All arabinosyl residues have been assigned the furanose ring form, and all other sugars the pyranose ring form.

[6] Either 3-glucose or 3-mannose is consistent with relative retention time and mass spectra data, but the peak at later ages is too large relative to total wall mannose content to be primarily 3-mannose.

TABLE III

Summary of Cotton Fiber Cell Wall Composition During Development

The first line of figures represents the percent by weight of that component as taken from the best fit line of plotted data points, the second line (italics) of figures represents the g x 10^{10} of component per mm fiber length. ND = not determined. Numbers in parentheses refer to the totaled percent of analyzed components under conditions where not all components were determined. Data refer only to plant-grown fibers.

COMPONENT	CELL WALL AGE (DPA)										
	5	8	10	12	14	16	18	20	22	25	29
Cellulose	9	10	23	24	25	31	74	82	87	90	94
	3	*3*	*6*	*9*	*14*	*34*	*131*	*201*	*292*	*484*	*1043*
Neutral Sugars	33	25	25	20	17	14	12	10	9	7	4
	11	*7*	*7*	*7*	*9*	*16*	*21*	*24*	*30*	*37*	*44*
Uronic Acids	10	16	22	21	16	9	3	2	2	ND	ND
	4	*5*	*6*	*8*	*9*	*10*	*5*	*5*	*6*		
Protein	40	24	22	22	25	21	3	3	ND	ND	ND
	14	*7*	*6*	*8*	*14*	*23*	*5*	*7*			
Ash	ND	3	3	2	11	11	ND	9	ND	ND	ND
		1	*1*	*1*	*6*	*12*		*22*			
Total Cell Wall	(91)	78	95	89	94	86	(92)	106	(98)	(97)	(98)
	34	*29*	*28*	*36*	*54*	*111*	*177*	*244*	*335*	*539*	*1110*

the thickness of the wall begins to increase, and during the period from 12 to 16 DPA increases in essentially all components contribute to the increase in total wall thickness. After 16 DPA, it is clear that deposition of cellulose (and to a much lesser extent, increase in noncellulosic glucose) are the only events which contribute to the continuing, great increase in wall thickening.

The data on cellulose deposition in the fiber wall point out two curious facts. The first is evident from the plot of the rate of cellulose deposition/unit length of fiber (Fig. 3B) which shows that the rate sharply rises at the onset of secondary wall deposition, declines, and then rises again. The second observation is that fiber elongation continues with little change in rate for at least 4 days after the first rise in the rate of cellulose deposition. This is curious because it seems that such an increase in deposition should cause an abrupt cessation of elongation. However, a similar observation, recently made by Benedict *et al.* (11) and Schubert *et al.* (44) for other varieties of cotton, revealed that it is not until the second rise in rate of cellulose deposition occurs that the fiber ceases to elongate. It is possible that the microfibrils continue to be deposited in a random or transverse orientation during this first phase of deposition and in this way do not limit cell extension; if so, secondary wall deposition might be more correctly defined as that deposition occurring during the time of distinct increase in birefringence at approximately 20 DPA. The other possibility is that the cells at this stage are elongating solely by tip growth and are not limited by deposition which occurs behind the tip region. There seem to be varietal differences in cotton regarding the capacity for elongation after the beginning of secondary wall deposition (11). In view of the commercial value of cotton fibers and the desirability of long lint fibers, this problem deserves further study.

The observation of a significant rise in noncellulosic glucan (which the methylation analyses indicate is, at least in part, 3-linked) during the later stages of cell elongation is of interest in light of the recent demonstration in our laboratory (17, 21) of a highly active β-(1→3)-glucan synthetase in these fibers. Although it is premature to speculate about the functional and/or structural role of this glucan, it is of interest that it increases in content concomitant with the earliest stages of secondary wall cellulose deposition and toward the end of elongation. Three-linked glucans are not thought to be common components of primary cell walls of higher plants. It remains to be determined whether this glucan is a true structural component of the wall or whether it serves as a reserve polymer, possibly for the biosynthesis of secondary wall cellulose. Callose is apparently mobilized as a reserve material in pollen (48), and there are other reports in the literature which implicate turnover of noncellulosic glucans of cell walls (14, 23, 31, 34). Callose has also been observed to be deposited behind the tip of growing pollen tubes (18), and it has been suggested that it serves to strengthen the tube wall (48), although the possibility that it turns over has apparently not been explored.

The question of turnover of all of the wall components of the cotton fiber merits further study since calculations based on the decrease in uronic acids and protein between 16 and 18 DPA lead to the conclusion that these components are partially removed from the wall during this time. It is clear that the patterns of cell wall synthesis (and probably also degradation) are undergoing great shifts at this period, and studies on the regulation of this transition period could help to provide a better general understanding of cell wall development in plants.

The results of both the compositional analyses and the preliminary methylation analyses permit us to compare the cotton fiber cell wall with some other primary cell walls as reported in the literature. Inasmuch as the primary wall of the cotton fiber is not a static structure, such comparisons are to a certain extent dependent upon the age of the fiber. A compositional comparison of the cotton fiber cell wall at 10 DPA (approximate midphase of elongation) with some other primary cell walls is shown in Table IV. All data used for comparison were obtained from cell walls of cultured cells, and the monocotyledons represented are all grasses. Wheat and rye grass cell walls have been shown to be representative of monocotyledon (grass) walls (15,) all of which are characterized by a high neutral sugar content, a low cellulose content, a very low content of hydroxyproline, and by an arabinose to xylose ratio approximating unity. Rye grass (endosperm cells) are, however, somewhat different from other monocot primary cell walls, in having higher levels of noncellulosic glucan which has been shown to be α-(1 → 3)-β-(1 → 4)-linked (46, 47). It is clear from Table IV that the cotton fiber cell wall at 10 DPA shows a greater similarity in composition to the walls of the dicot sycamore maple (representative of other dicot primary cell walls examined [56]) than to those of the monocots, but it resembles even more closely that of cell walls of cultured cells of the gymnosperm, Douglas fir.

The results of the preliminary methylation analyses of unfractionated walls provide further support for the similarity of the cotton fiber cell wall to the cell walls of cultured cells of dicots and the gymnosperm. Data of Burke *et al.* (15) indicate that the major hemicellulosic component of primary walls of cells of dicots and of Douglas fir is a (galacto)-xyloglucan, whereas that of the cell walls of the grasses in culture is an arabinoxylan. The cotton fiber walls contain significant amounts of 4-linked glucose, 4,6-linked glucose, terminal xylose, 2- or 4-linked xylose, and terminal galactose residues indicative of a (galacto)-xyloglu-

TABLE IV

Composition by Weight Percent of Cell Walls from Various Plant Sources

	Wheat[1]	Rye Grass[1]	Sycamore[1] maple	Douglas fir[1]	Cotton fiber[2]
Total neutral sugars	63.1	73.7	49.8	37.2	25.3
Rhamnose	0.6	0.6	3.1	2.5	1.9
Fucose	0.1	0	1.3	1.0	0.3
Arabinose	20.7	20.3	21.0	12.7	8.2
Xylose	24.8	19.8	7.6	5.0	2.6
Mannose	1.0	0.1	0.3	0.5	1.0
Galactose	12.0	5.9	12.8	7.6	4.3
Glucose (non-cellulosic)	3.9	27.0	3.7	7.9	7.0
Cellulose	14.0	13	23	22	23
Uronic acids	12	7	13	18	22
Protein	11	7	10	18	22
Hydroxyproline	0.14	<.05	2.0	0.4	0.3

[1]Taken from the data of Burke et al (15)

[2]From 10 DPA, plant grown fibers

can structure, and lack branched xylosyl residues indicative of the arabinoxylan characteristic of monocot cell walls. The preponderance of 5-linked arabinose in the cotton fiber walls is another similarity shared with the Douglas fir and may indicate the presence of a polymer with an α-(1 → 5)-arabinan backbone similar to those reported from a variety of plant tissues (6, 7). Although the amount of hydroxyproline is low in the cotton fiber cell walls, the presence of some 2-linked arabinosyl residues in these preparations could indicate such arabinosides (30). Additional studies may show that the wall structure of cotton fiber cell walls is indeed representative of dicots, and as such elongating cotton fibers might be well suited to serve as a model system for elongation studies. A critical assessment of the mode of growth of these fibers will also be necessary before detailed elongation studies can be properly interpreted; although it appears unlikely, if growth proceeds from the tip alone, then these observed changes in composition may be more properly attributed to changes associated with wall maturation rather than elongation. We stress that the changes in composition reported here should for the present be interpreted only as changes correlated in time with the elongation process. Much more detailed study will be necessary to determine whether any or all of these compositional changes are fundamental to the mechanism of cell wall growth in this system.

Our data show that cell walls from fibers cultured *in vitro* are in many respects quite similar to those of fibers grown on the plant. The major similarities are the like compositions of the cell walls and that changes in composition occur in the same developmental sequence on the plant and in culture. The entire developmental sequence seems to be compressed in time in culture, and the final length of fibers is reduced by about half. Since an earlier onset of secondary wall deposition may limit fiber length, this may account for the shorter final fiber length. The biochemical and developmental similarities of walls in culture should make this system useful for *in vivo* labeling studies designed to examine turnover of wall components and perhaps also for studying the effects of hormones on the elongation process.

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